COMPARATIVE VIRULENCE OF *Beauveria bassiana* (Bals.) Vuill. AND *Metarhizium anisopliae* (Metchnikoff) Sorokin TO *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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ABSTRACT

The invasive pest, fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), was first reported to infest corn fields in the Philippines in 2019. In search for biological control agents against this pest, this study evaluated the bioefficacy of entomopathogenic fungi under laboratory conditions. The different life stages of *S. frugiperda* were exposed to *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metchnikoff) Sorokin. Larval hatch from treated eggs was reduced by the entomopathogenic fungi at 1 x 10^9 conidia/ml. Hatchability of treated eggs was significantly reduced in *B. bassiana* and *M. anisopliae* by 26.84% and 46.48%, respectively. Lethal infection to the larval instars (1^{st} to 6^{th}) ranged from 23.64 to 97.42% in *B. bassiana* and 23.13 to 61.33% in *M. anisopliae* at 10 days after treatment. *B. bassiana* was more virulent to 1^{st} instar larvae, however, the two entomopathogenic fungi had similar degree of virulence to eggs, 2^{nd} to 6^{th} larval instars, prepupae, and pupae. Lower LC50 values were calculated for *B. bassiana* (0.0642 to 9.43 x 10^8 conidia/ml) than in *M. anisopliae* (1.61 x 10^6 to 6.13 x 10^9 conidia/ml). Mean time to larval mortality ranged from 4.59 to 7.46 days in *B. bassiana* and 4.06 to 7.79 days in *M. anisopliae*. However, the entomopathogenic fungi inflicted low mortality in treated prepupae and did not affect the pupal characteristics such as length, width, and weight. No significant difference was observed in the adult emergence of treated pupae. However, abnormalities in adults such as reduced size of wings and deformities were observed. These findings provided evidence on the effect of *B. bassiana* and *M. anisopliae* to the different life stages of *S. frugiperda*, hence, can be used as alternative to chemical insecticides against *S. frugiperda*.

Key words: Fall armyworm, entomopathogenic fungi, biological control, life stages, pathogenicity

INTRODUCTION

Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a biosecurity threat to agriculture due to its migratory behavior and wide host range. Larval feeding results in extensive defoliation and corn ear damages (Capinera 2020) with possible yield reduction of 5 to 20% if 0.2 to 0.8 larvae attack each sweet corn plant during late whorl stage as reported in Florida (Marenco et al. 1992). This invasive pest is native to tropical and subtropical regions in America (Food and Agriculture Organization 2018). *S. frugiperda* is also considered a serious insect pest since it reproduces in several generations per year and the adult moth can fly up to 100 km per night. It was presumed to be introduced by trade and migration to Africa and Asia causing severe damage to corn fields. This notorious pest was first reported in the Philippines in 2019 with confirmed incidences in 17 municipalities in 10 provinces (Navasero et al. 2019). The insect pest is reported to cause economic damage to more than 80 plant species (Food and Agriculture Organization 2018). All growth stages of corn are being infested with larval preference in early growth stages of corn. Management recommendations against *S. frugiperda* are being determined by FAO in coordination with member

countries. Insecticide application, varietal identification, cultural practices, and augmentation of natural enemies are promising strategies to mitigate this pest. Numerous eggs, larvae, and pupal parasitoids, and predators attack *S. frugiperda* (Capinera 2020; Koffi et al. 2020; Shylesha et al. 2018). *Spodoptera frugiperda* nuclear polyhedrosis virus (NPV), fungi, protozoa, nematodes, and bacteria are known as entomopathogens of this pest (Business Queensland 2020; Capinera 2020). Entomopathogenic fungi including *Nomuraea rileyi* (Shylesha et al. 2018), *Entomophaga aulicae* and *Erynia radicans* (Capinera 2020) were reported to cause epizootics to this pest.

Beauveria and *Metarhizium* species are common entomopathogenic fungi with broad spectrum of pathogenicity against various insect pests. *M. rileyi* was pathogenic to *S. litura* (Liu et al. 2019), *S. exigua* (Montecalvo and Navasero 2020), and *S. frugiperda* (Montecalvo and Navasero 2021). Asi et al. (2013) also noted the virulence of *M. anisopliae*, *Isaria fumosorosea*, and *B. bassiana* against *S. litura*. Ramanujam et al. (2020) assayed entomopathogenic fungi against 2nd instar larvae of *S. frugiperda* and discovered that *M. anisopliae* ICARNBAIR Ma-35 and *B. bassiana* ICAR-NBAIR-Bb-45 as potential biological control agents in corn based on their efficacy in laboratory and field trials with minimum infestation levels of *S. frugiperda* and considerable increase in the yield than the untreated control. Akutse et al. (2019) and Ramos et al. (2020) also assayed isolates of *B. bassiana* and *M. anisopliae* against *S. frugiperda*. In the Philippines, *M. rileyi* isolate was infective against the 3rd instar larvae of *S. exigua* with 100% mortality at 1 x 10^7 and 1 x 10^8 conidia/ml at 7 days after treatment (Montecalvo and Navasero 2020). The same isolate cross infected *S. frugiperda* causing up to 100% larval mortality at 7 days after treatment with LC50 of 1.44 x 10^5 to 9.36 x 10^8 conidia/ml and mean time to death of 4.51 to 8.89 days (Montecalvo and Navasero 2021).

Due to the biological control potential of entomopathogenic fungi, bioassays were conducted to evaluate the potential of two entomopathogenic fungi against *S. frugiperda*. This paper elucidates the effect of *B. bassiana* and *M. anisopliae* on the different life stages of *S. frugiperda*. Likewise, the biocontrol efficacy of these entomopathogenic fungi were further assessed through calculation of lethal dose and time estimates.

MATERIALS AND METHODS

Laboratory rearing of *S. frugiperda.* Field collected larvae of *S. frugiperda* from Barangay Patel, Gonzaga, Cagayan, Philippines were reared at the Biocontrol Laboratory of National Crop Protection Center, College of Agriculture and Food Science, University of the Philippines Los Baños, Laguna, Philippines. Neonates were fed with fresh leaves of a native variety of corn until the desired stages of larvae were obtained. Bioassays were conducted when the appropriate age/instar of test insects was reached.

Preparation of fungal cultures and spore suspensions of *B. bassiana* and *M. anisopliae.* The *B. bassiana* isolate was sourced from the Department of Agriculture (DA)-Bureau of Plant Industry, Malate, Manila, Philippines. The isolate of *M. anisopliae* was obtained from the DA-Regional Crop Protection Center CALABARZON, Los Baños, Laguna, Philippines. Molecular characterization of these isolates were done to confirm their identity. These entomopathogenic fungi were revived and subcultured in PDA for at least 7 days. Conidia were harvested from the fungal cultures and suspended in 0.1% Tween 80 solution. The number of conidia in the suspension were counted using Neubauer Improved haemacytometer. Various conidial concentrations were prepared for the bioassays by diluting the stock in 0.1% Tween 80 solution.

Bioefficacy of *B. bassiana* and *M. anisopliae* against eggs of *S. frugiperda.* Egg masses were treated with conidial concentration (1 x 10^9 conidia/ml) of *B. bassiana* and *M. anisopliae*. In the field, egg-masses are laid in the netter surfaces covered with tufts of hair from the anal segment of the female abdomen which provide protection to the eggs from natural enemies. As such, the concentration used

in this bioassay was the highest in the range. Control set-up was treated with 0.1% Tween 80 solution. Treated egg masses were incubated in a Petri plate with moistened cotton balls. Corn leaves were surface sterilized with sodium hypochlorite (0.5%v/v) followed by washing twice with sterile distilled water (Asi et al. 2013). Surface sterilized corn leaves were provided inside the Petri plates daily. Each treatment was replicated with four egg masses per treatment. The number of eggs in each mass was counted under dissecting microscope. Neonates hatched from treated egg masses were recorded and removed daily until 7 days after treatment. Hatchability was computed using the formula: percent hatchability = [(number of eggs hatched) / (total number of eggs in the mass)] x 100%.

Dose-mortality assays of *B. bassiana* and *M. anisopliae* on different larval instars of *S. frugiperda*. Conidial concentrations (1 x 10^5 to 1 x 10^9 conidia/ml) of *B. bassiana* and *M. anisopliae* were used in the bioassays and each treatment was replicated three times. A total of 10 larvae of *S. frugiperda* was used per instar in each replicate. For 1st and 2nd instar larvae, 10 larvae were initially cultured per Petri plate which later on cultured individually upon reaching 3rd instar. The other larval instars (3rd to 6th) were single cultured per Petri plate.

Young corn leaves (5-cm length) were surface sterilized as described above. Conidial concentrations were sprayed to both sides of the corn leaves using a mist sprayer. Tween 80 (0.1%) was applied to corn leaves for the control set-up. Four treated corn leaves were fed to *S. frugiperda* larva/e in a Petri plate. Each Petri plate was provided with moistened cotton and sealed with Parafilm. Fresh corn leaves that were surface sterilized were fed to the larvae daily. Larval mortality was observed daily. Mycosis of cadavers was confirmed in blotter set-up. Cadavers were dipped in 1% sodium hypochlorite for 1 min and washed twice in sterile distilled water for 1 min. The cadavers were dried in sterile filter paper and placed in a microscope slide inside a Petri plate lined with moistened filter paper.

Percentage mortality was corrected using the equation: $M\ (\%) = [(t - c) / (100 - c)] \times 100$, where: M = corrected mortality; c = percentage mortality in controls; t = percentage mortality in treatments (Abbott 1925). Mean time to death was calculated using the formula: mean time to death (d) = $[(x_1y_1)+(x_2y_2)+(x_ny_n)]$ / total mortality, where: x = number of larvae died on a given day; y = number of days of which the observation was made considering the time when the trial was initiated (El-Hawary and Abd El-Salam 2009).

Bioefficacy evaluation of *B. bassiana* and *M. anisopliae* on *S. frugiperda* prepupae and pupae. This bioassay was conducted following the methodology of Asi et al. (2013). Prepupae and 2-day old pupae of *S. frugiperda* were surface sterilized with 0.5% (v/v) sodium hypochlorite. Prepupae and pupae were subsequently washed in two washes of sterile distilled water.

Conidial concentration (1 x 10^8 conidia/ml) of *B. bassiana* and *M. anisopliae* was tested against prepupae. The pupae, on the other hand, were dipped in conidial concentrations (1 x 10^5 to 1 x 10^9 conidia/ml) of *B. bassiana* and *M. anisopliae* for 2 min with gentle shaking. Each treatment was replicated thrice with 10 prepupae and pupae per replicate. Control set-up were dipped in 0.1% Tween 80 solution. Treated prepupae and pupae were incubated in Petri plates with moistened cotton. Pupal length, width, and weight were recorded. Likewise, adult emergence and abnormalities in adults were noted.

Statistical analyses. One-way ANOVA was performed to analyze the effect of entomopathogenic fungi to eggs, prepupae, and pupae. Treatment means were compared using Tukey's honestly significant difference (HSD) test. The comparative effect of *B. bassiana* and *M. anisopliae* to larval mortality and mean time to larval mortality was compared by t-test ($P \leq 0.05$) (PROC TTEST). Lethal concentration (LC) estimates were calculated using PriProbit ver 1.63.

RESULTS AND DISCUSSION

Mycosis of *S. frugiperda* eggs. The entomopathogenic fungi *B. bassiana* and *M. anisopliae* caused lethal infection to different growth stages of *S. frugiperda*. Among the life stages, eggs and larvae were susceptible to fungal infection. The entomopathogenic fungi exhibited ovicidal activity. Exposure to conidial suspension (1 x 10^9 conidia/ml) of these entomopathogenic fungi affected the larval emergence from treated egg masses (Fig. 1). Hatchability of eggs was reduced significantly in *B. bassiana* and *M. anisopliae* by 26.84% and 46.48%, respectively. Fungal growth covered the egg masses at 3 to 4 days after treatment.

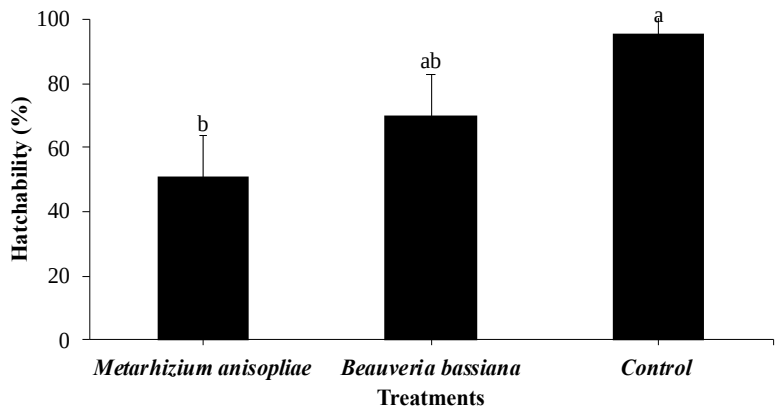

Fig. 1. Hatchability of *Spodoptera frugiperda* egg masses as affected by conidial suspension (1 x 10^9 conidia/ml) of *Metarhizium anisopliae* and *Beauveria bassiana.* Means are significant at $P< 0.05$.

The mortalities obtained in this study using 1 x 10^9 conidia/ml were lower compared to the results of Akutse et al. (2019) using 1 x 10^8 conidia/ml. Assays of *B. bassiana* and *M. anisopliae* isolates revealed that *M. anisopliae* caused mortalities up to 87.00% and 96.50% against *S. frugiperda* eggs and neonate larvae, respectively; whereas *B. bassiana* caused moderate mortality (30.00%) to 2nd instar larvae (Akutse et al. 2019). These results were in contrast with the findings of Ramanujam et al. (2020) wherein no mortality was recorded on *S. frugiperda* eggs treated with 1 x 10^8 conidia/ml of *M. anisopliae* ICAR-NBAIR Ma-35 and *B. bassiana* ICAR-NBAIR Bb-45. On the other hand, various conidial concentrations (1 x 10^5 to 1 x 10^8 conidia/ml) demonstrated the virulence of *M. anisopliae*, *Isaria fumosorosea*, and *B. bassiana* on *S. litura* eggs, with 48.19 to 71.56% egg mortality (Asi et al. 2013).

Effect on different larval instars of *S. frugiperda.* Different conidial concentrations of *B. bassiana* and *M. anisopliae* were assayed against 1st to 6th larval instars of *S. frugiperda*. Dead larvae treated with entomopathogenic fungi were firm and hard. Mycosis of *S. frugiperda* larvae confirmed fungal infection (Fig. 2 and Fig. 3). Those infected with *B. bassiana* showed white muscardine disease with white fungal growth covering the cadaver. Mummified cadavers infected with *M. anisopliae* had green fungal growth indicating the green muscardine disease.

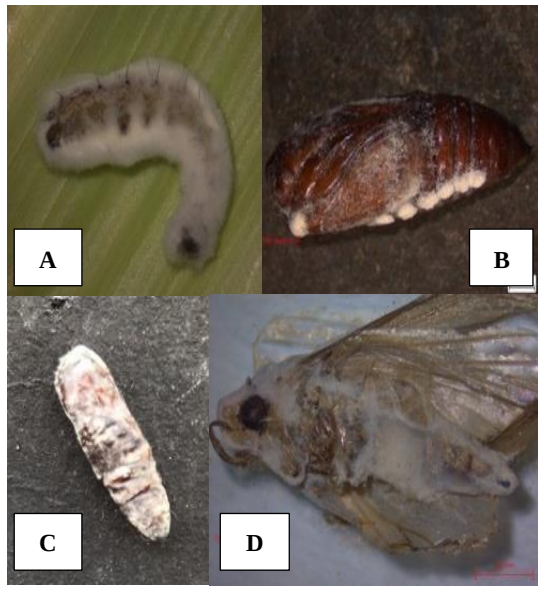

Fig. 2. Mummification of *Spodoptera frugiperda* due to *Beauveria bassiana*: larva (a) and pupa (b and c) at 10x magnification, and adult at 8x magnification (d).

Fig. 3. Mycosis of *Spodoptera frugiperda* larvae at 10x magnification (a) and prepupae (b) due to exposure to conidial concentrations of *Metarhizium anisopliae*.

Based on mean mortality per instar, fungal infection initiated 1-2 days after treatment and significantly increased 4 days after treatment (Fig. 4). However, Ramos et al. (2020) recorded varying initiation of fungal infection in *S. frugiperda* treated with *B. bassiana* and *M. anisopliae*. Infection due to *B. bassiana* on 2nd and 4th larval instars initiated 4 days after treatment, in contrast with *M. anisopliae* wherein infection initiated 3 days after treatment. El Husseini (2019), on the other hand, recorded death of *S. littoralis* due to *M. anisopliae* at 4 days after treatment.

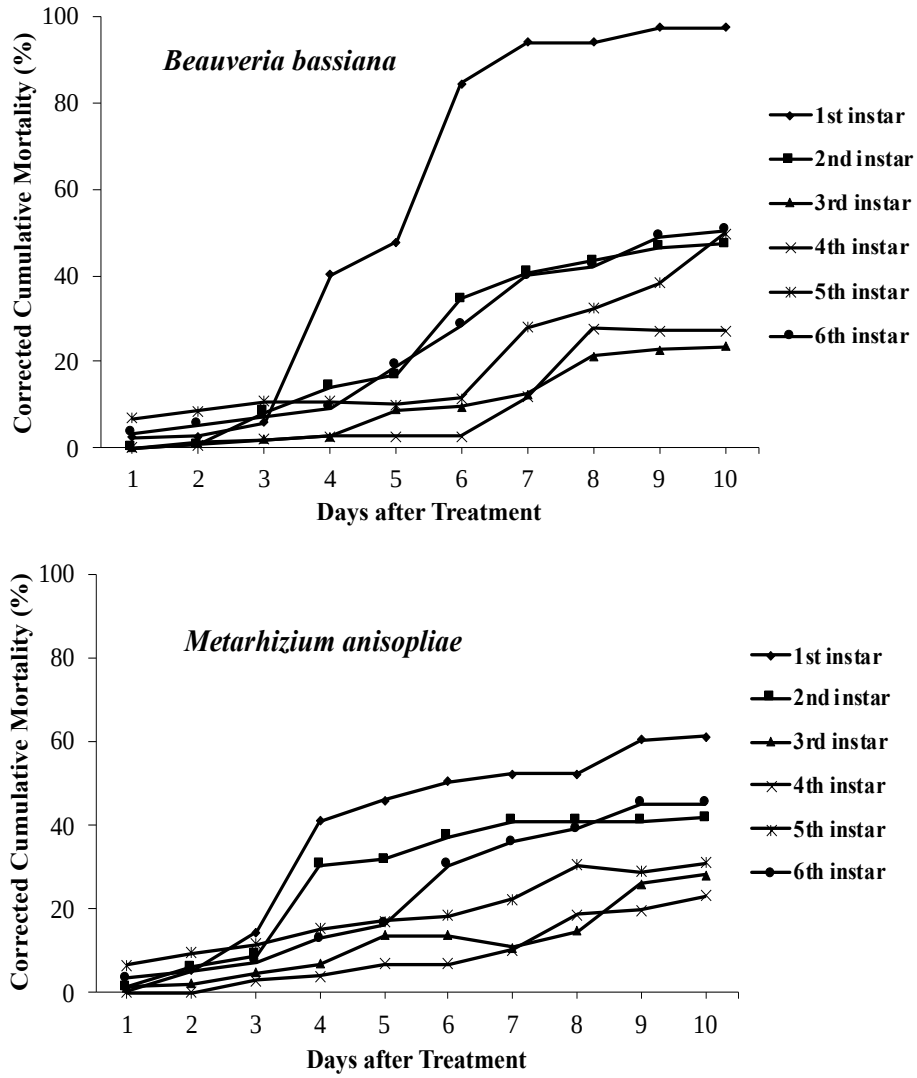

Fig. 4. Corrected cumulative mean mortality of larval instars of *Spodoptera frugiperda* treated with *Beauveria bassiana* (top) and *Metarhizium anisopliae* (bottom).

In this research, early larval instars specifically 1st instar was the most susceptible to both entomopathogenic fungi. *B. bassiana* (97.42%) was more virulent than *M. anisopliae* (61.33%) in inflicting lethal infection to 1st instar larvae of *S. frugiperda* at 10 days after treatment (Fig. 5). However, these entomopathogenic fungi have similar degree of pathogenicity against 2nd to 6th larval instars with mortalities lower than 50%. Infection in 2nd to 6th larval instars caused by *B. bassiana* was 23.64 to 50.37% while 23.13 to 45.33% in *M. anisopliae*. Lower LC50 was calculated for *B. bassiana* (0.0642 to 9.43 x 10^8 conidia/ml) than in *M. anisopliae* (1.61 x 10^6 to 6.13 x 10^9 conidia/ml) (Table 1). Due to high infection at 10 days after treatment, the computed LC50 was very low (0.0642 conidia/ml) particularly in 1st instar larvae infected with *B. bassiana*. Mean time to larval mortality ranged from 4.59 to 7.46 days in *B. bassiana* and 4.06 to 7.79 days in *M. anisopliae* (Fig. 6). Based on t-test, the entomopathogenic fungi had similar calculated mean time to larval mortality for 1st, 3rd, and 4th larval instars. However, *B. bassiana* caused delayed infection in 2nd, 5th, and 6th larval instars of about 1.30 to 1.82 days.

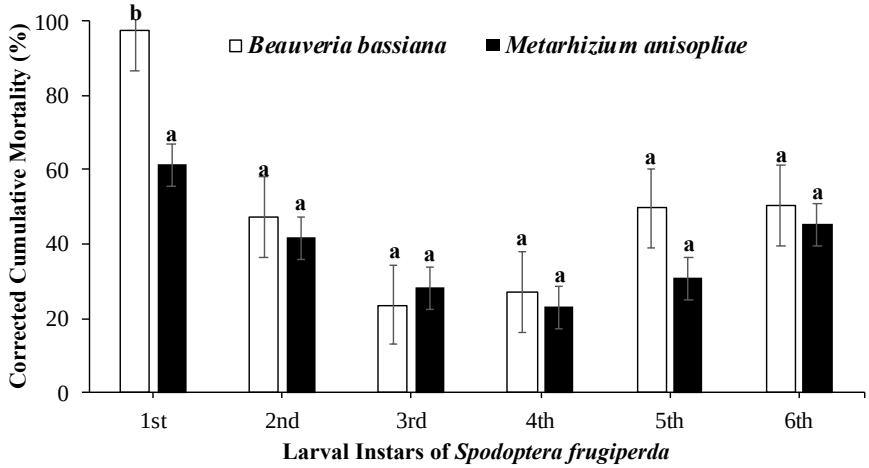

Fig. 5. Comparative efficacy of *Beauveria bassiana* and *Metarhizium anisopliae* in inflicting mortality to various larval instars of *Spodoptera frugiperda* at 10 days after treatment. Pairs of columns with the same letters are not significantly different by *t*-test ($P< 0.05$).

Table 1. Lethal concentration estimates of *Beauveria bassiana* and *Metarhizium anisopliae* against *Spodoptera frugiperda*.

Entomopathogenic Fungi	Larval Instar	Lethal Concentration (LC) 50 (conidia/ml) (95% FL)	Regression Equation (y= a+bx)	Chi-square
Beauveria bassiana				
	1st	0.0642	Y=0.306 + 0.256x	1.21
	2nd	1.67 x 10^7	Y=-2.495 + 0.345x	5.76
	3rd	4.48 x 10^8	Y=-6.302 + 0.728x	5.81
	4th	9.43 x 10^8	Y=-11.637 + 1.297x	7.91
	5th	2.72 x 10^7	Y=-2.506 + 0.337x	4.23
	6th	3.01 x 10^7	Y=-1.507 + 0.202x	12.48
Metarhizium anisopliae				
	1st	1.61 x 10^6	Y=-3.595 + 0.579x	4.20
	2nd	6.32 x 10^7	Y=-6.416 + 0.822x	13.08
	3rd	1.95 x 10^9	Y=-4.998 + 0.538x	0.62
	4th	6.13 x 10^9	Y=-2.974 + 0.304x	13.62
	5th	9.10 x 10^8	Y=-56.492 + 6.306x	6.02
	6th	5.07 x 10^7	Y=-0.847 + 0.110x	2.54

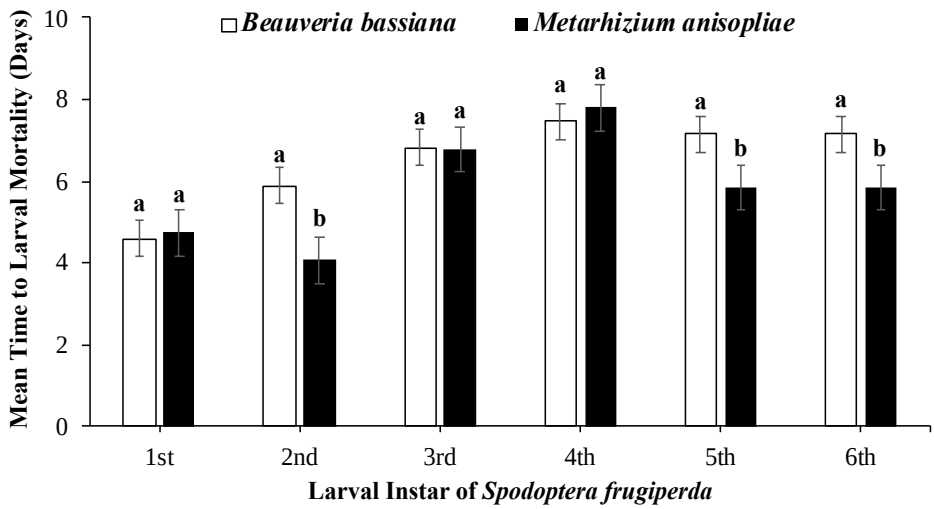

Fig. 6. Mean time to larval mortality (days) of *Spodoptera frugiperda* influenced by lethal infection due to *Beauveria bassiana* and *Metarhizium anisopliae*. Pairs of columns with the same letters are not significantly different by *t*-test ($P< 0.05$).

The vulnerability of early instars to fungal infection particularly the first instar larvae with highest mortality and fastest infection was also observed by Montecalvo and Navasero (2021). The early larval instars (1st–3rd) of *S. frugiperda* were more susceptible to *M. rileyi* than late larval instars (4th–6th). Higher conidial concentrations caused higher and faster rate of larval mortality than lower conidial concentrations. Likewise, *M. anisopliae* ICAR-NBAIR Ma-35 and *B. bassiana* ICAR-NBAIR Bb-45 isolates were discovered with promising bioefficacy against 2nd instar larvae of *S. frugiperda* (Ramanujam et al. 2020). *B. bassiana* ICAR-NBAIR Bb-45 caused 64.3% mortality and *M. anisopliae* ICAR-NBAIR Ma-35 with 67.8% mortality. Larvae of *S. litura* was also susceptible to *B. bassiana* and *M. anisopliae* applied at 1 x 10^7 conidia/ml, which induced 27.30 to 59.06% mortality and 14.28 to 41.16% mortality, respectively, at 10 days after treatment (Asi et al. 2013).

Higher LC50 values were obtained in this study as compared with the results of Ramanujam et al. (2020) wherein calculated LC50 was 1.1×10^7 spores/ml and LT50 of 3.59 days in *M. anisopliae* ICAR-NBAIR Ma-35 whereas *B. bassiana* ICAR-NBAIR Bb-45 showed LC50 of 1.9×10^7 spores/ml and LT50 of 3.68 days against 2nd larval instar of *S. frugiperda*. Their field trials further confirmed the bioefficacy of both entomopathogenic fungi with reduction in *S. frugiperda* infestation further resulting in increase in yield.

Resistance to fungal infection as the larvae mature may be attributed to the composition of the larval integument that allowed effective penetration of the fungus resulting in higher mortality in early larval instars (Bosa et al. 2004). Molting may be the reason for loss of inoculum (Meekes 2001), hence, lower chances of fungal infection, although it does not always result in an escape from fungal infection.

Effect on prepupae and pupae of *S. frugiperda.* The entomopathogenic fungi at 1 x 10^8 conidia/ml caused low mortality in prepupae with only 3.33% in *B. bassiana* and 20.00% in *M. anisopliae* (Table 2, Fig. 2b-d, and Fig. 3b). Pupal characteristics such as length, width, and weight did not vary significantly between fungal-treated and in control.

Table 2. Effect of *Beauveria bassiana* and *Metarhizium anisopliae* on mortality, adult emergence, and pupal characteristics of treated *Spodoptera frugiperda* prepupae.

Treatments	Mortality* (%)	Unemerged Pupae** (%)	Pupal Length** (mm)	Pupal Width** (mm)	Pupal Weight** (g)
Beauveria bassiana	3.33 ± 3.33ab	23.33 ± 18.56ab	15.63 ± 0.18a	4.68 ± 0.05a	0.19 ± 0.00a
Metarhizium anisopliae	20.00 ± 10.00a	40.00 ± 20.00a	15.31 ± 0.18a	4.67 ± 0.07a	0.19 ± 0.01a
Control	0.00 ± 0.00b	7.41 ± 7.41a	15.63 ± 0.18a	4.72 ± 0.02a	0.19 ± 0.00a

* Values represent means ± SE. Means within the same column followed by a different letter are significant at P<0.05, HSD test.
**Means in same column having same letter are not significantly different at P<0.05, HSD test.

The pupae of *S. frugiperda* was also exposed to the entomopathogenic fungi (Table 3). Adult emergence was not significantly different among conidial concentrations of *B. bassiana* and *M. anisopliae*. Reduced size of wings and deformities were noted in adults emerged from treated pupae. Abnormalities in adults of *S. frugiperda* were recorded in 11.54 to 24.13% and 7.14 to 27.59% of treated pupae with *B. bassiana* and *M. anisopliae*, respectively. These findings suggest that pupae are not susceptible to fungal infection. However, it should be noted that fungal infection caused deformities to adults which may affect its mating and oviposition behavior as well as reproduction.

Similarly, pupae of *S. litura* were less susceptible to entomopathogenic fungi, however, adult emergence was delayed in fungal treated pupae (Asi et al. 2013). Malformations in emerged adults from treated pupae were observed with reduced wings and body size making them unable to fly and eventually die without mating. The findings in this study, however, are contradictory to the observations of Ekesi et al. (2002) wherein pupae treated with fungal pathogens result in lower adult emergence. Low mortality in prepupae and pupae can be attributed to the shorter period for fungal infection to occur. In addition, the thick and sclerotized cuticle of pupae serves as barrier to fungal infection, hence, this life stage is seldom being attacked by fungal pathogens (Hajek and St. Leger 1994).

Table 3. Effect of *Beauveria bassiana* and *Metarhizium anisopliae* on adult emergence of *Spodoptera frugiperda* pupae.

Conidial Concentration (conidia/ml)	*Beauveria bassiana*		*Metarhizium anisopliae*	
	Adult Emergence* (%)	Abnormal Adults** (%)	Adult Emergence* (%)	Abnormal Adults** (%)
10^5	86.67 ± 3.33a	11.54 ± 5.77e	93.33 ± 6.67a	7.14 ± 6.67e
10^6	96.67 ± 3.33a	20.69 ± 0.00b	86.67 ± 8.82a	15.38 ± 8.82d
10^7	93.33 ± 3.33a	17.86 ± 5.77c	96.67 ± 3.33a	27.59 ± 6.67a
10^8	90.00 ± 0.00a	14.81 ± 3.33d	90.00 ± 5.77a	25.93 ± 8.82b
10^9	96.67 ± 3.33a	24.13 ± 3.33a	93.33 ± 6.67a	21.43 ± 5.77c
Control	96.67 ± 3.33a	3.45 ± 3.33f	90.00 ± 5.77a	3.70 ± 3.33f

* Values represent means ± SE. Means in same column having same letter are not significantly different at P<0.05, HSD test.
**Means within the same column followed by a different letter are significant at P<0.05, HSD test.

This study corroborated with several bioassays denoting the differences in the virulence of the entomopathogenic fungi depending on the origin of strain. The strains of our entomopathogenic fungi had varying bioefficacy with the other strains reported to inflict mycosis to *Spodoptera* species. Genetic diversity and insect cuticle characteristics contribute to the differences in the pathogenicity of *M. rileyi* (Fronza et al. 2017). The enzymes that are secreted by the entomopathogenic fungi have a role in their virulence against insect pests, which suggest the differences in virulence of entomopathogenic fungi (Fang et al. 2005). The proteases of the fungal pathogens on the cuticle have damaging effects due to the structural importance and enzymatic accessibility of protein polymers in the cuticle (Hajek and St. Leger 1994). Fang et al. (2005) also observed that the overproduction of Bbchit1 contributed to the enhanced virulence of *B. bassiana* resulting in lower LC50 and LT50 of the transformants compared to the values for the wild-type strain.

The use of these entomopathogenic fungi (*B. bassiana* and *M. anisopliae*) in the integrated pest management of *S. frugiperda* is promising since these fungi can establish endophytically in maize plants (Ramos et al. 2020). Their colonization in maize plants caused 100% mortality on 2nd instar larvae, while 87% and 75% mortality were recorded on the 4th instar larvae treated with *B. bassiana* and *M. anisopliae*, respectively.

CONCLUSION AND RECOMMENDATION

The entomopathogenic fungi, *B. bassiana* and *M. anisopliae*, were pathogenic to various life stages of *S. frugiperda*. These entomopathogenic fungi affected the hatchability of treated eggs, caused larval and prepupal mortality, and abnormalities in adults emerging from treated pupae. Results also indicate the varying virulence of *B. bassiana* and *M. anisopliae*. *B. bassiana* was more pathogenic to 1st instar larvae. However, these entomopathogenic fungi have similar degree of virulence to eggs, 2nd to 6th instar larvae, prepupae, and pupae of *S. frugiperda*. Further tests will be conducted to assess the efficacy of these entomopathogenic fungi in greenhouse and field experiments, which can be measured by the degree of reduction in damage level and yield increase.

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